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LABORATORY INVESTIGATION

Effects of somatostatin on phosphate transport: Evidence for the role of basal insulin

KAI LAU, JAY GUNTUPALLI, and BONNIE EBY

Division of Nephrology, Department of Internal Medicine, The University of Michigan, Ann Arbor, Michigan

Effects of somatostatin on phosphate transport: Evidence for the role of basal insulin. Previous studies suggest that normoglycemic hyperinsulinemia is associated with enhanced proximal tubule and overall renal phosphate (PO_4) reabsorption. It is unclear whether the basal level of insulin also regulates PO_4 transport. Furthermore, the role of parathyroid hormone, the associated antinatriuresis, and the distal nephron is uncertain. To examine these issues, clearance and recollection micropuncture studies were performed in volume-expanded parathyroidectomized rats. Infusion of des-Asn^5-D-Trp^8-D-Ser^{13}, a somatostatin analogue highly selective for insulin at 10 μg/kg/hr, decreased plasma insulin (12.6 to 2.8 μU/ml), and increased plasma glucose (93 to 140 mg%) and fractional excretion (FE) of sodium (Δ = 2.5%). The clearance of PO_4 (118 to 297 μl/min) and FE_{PO_4} (2.4 to 6.8%) was increased. Continued somatostatin infusion produced a sustained phosphaturia whereas the addition of insulin (0.10 U/kg/hr) abolished the phosphaturic effects. Separate loading with saline and glucose to achieve comparable ΔFE_{Na} (2.9%) and Δplasma glucose (52 mg%) did not increase PO_4 excretion. Between basal and experimental phases, there was no difference in plasma ultrafiltrable PO_4, GFR, or segmental fluid reabsorption. While fractional delivery of $PO_4(FD_{PO_4})$ was unchanged in the control, somatostatin increased FD_{PO_4} to late proximal (50 vs. 32%), early distal (28.2 vs. 13.4%), late distal tubules (11.8 vs. 6.4%), and to the urine (8.5 vs. 4.9%). These data indicate that *(1)* insulin deficiency, as produced by somatostatin infusion, impairs tubular PO_4 reabsorption, suggesting an important role for the basal level of insulin in renal PO_4 homeostasis; *(2)* the phosphaturia of somatostatin is independent of the concomitant natriuresis, hyperglycemia, PTH, and changes in plasma PO_4; and *(3)* insulinopenia reduces PO_4 transport in the proximal convoluted tubule and the distal nephron.

Effets de la somatostatine sur le transport du PO_4: Preuve d'un rôle de l'insuline basale. Des études antérieures suggèrent que l'hyperinsulinémie normoglycémique est associée à une augmentation de la réabsorption rénale de phosphate (PO_4) globale et dans le tubule proximal. On ne sait pas bien si le niveau basal d'insuline régule également le transport de PO_4. En outre, le rôle de l'hormone parathyroïdienne, de l'antinatriurèse associée et du néphron distal est incertain. Afin d'examiner ces possibilités, des études de clearance et de microponction avec recollection ont été effectuées chez des rats parathyroïdectomisés en expansion volémique. La perfusion de des-Asn^5-D-Trp^8-D-Ser^{13}, un analogue de la somatostatine hautement sélectif pour l'insuline à 10 μg/kg/hr, a diminué l'insuline plasmatique (12.6 à 2.8 μU/ml), augmenté la glycémie (de 93 à 140 mg%) et l'excrétion fractionnelle (FE) de sodium (Δ = 2.5%). La clearance de PO_4 (118 à 297 μl/min) et FE_{PO_4} (2.4 à 6.8%) se sont élevées. Une perfusion prolongée de somatostatine a entraîné une phosphaturie persistante tandis que l'addition d'insuline (0.10 U/kg/hr) a aboli les effets phosphaturiants. Une charge séparée avec du soluté salé et du glucose pour obtenir un Δ FE_{Na} (2.9%) et un Δ glycémie (52 mg%) comparables n'a pas augmenté l'excrétion de PO_4. Entre les phases basale et expérimentale il n'y avait pas de différence dans le PO_4 ultrafiltrable, le GFR ni la réabsorption segmentaire de fluide. Tandis que la fraction délivrée de $PO_4(FD_{PO_4})$ était inchangée pendant le contrôle, la somatostatine a augmenté FD_{PO_4} aux tubules proximal tardif (50 contre 32%), distal précoce (28.2 contre 13.4%) et tardif (11.8 contre 6.4%), et à l'urine (8.5 contre 4.9%). Ces données indiquent que *(1)* la carence en insuline résultant d'une perfusion de somatostatine altère la réabsorption tubulaire de PO_4, suggérant un rôle important de la concentration de l'insuline de base dans l'homéostasie du PO_4; *(2)* la phosphaturie due à la somatostatine est indépendante de la natriurèse concomitante, de l'hyperglycémie, de la PTH, et des modifications du PO_4 plasmatique; *(3)* l'insulinopénie réduit le transport de PO_4 dans le tubule contourné proximal et le néphron distal.

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Previous studies in intact humans [1–2], dog [3], and rat [4] suggest that hyperinsulinemia is associated with enhanced renal PO_4 reabsorption, characterized by increased proximal tubule transport [3]. Because there was a concomitant antinatriuresis [2, 3] or antidiuresis [1], and because glucose was administered, it is uncertain as to the role played by endogenous parathyroid hormone (PTH), exogenous glucose load, and the changes in renal handling of sodium. Furthermore, it is not clear whether the antiphosphaturic phenomenon of hyperinsulinemia is mirrored by a phosphaturic effect of insulin deficiency. The objectives of the present investigation were to examine these issues and to evaluate tubular PO_4 transport in response to a reduction in plasma insulin levels.

Methods

Male Sprague-Dawley rats weighing 275 to 375 g were used. Parathyroidectomy (PTX) was performed and confirmed as previously described [5]. Both before and after PTX, the rats were fed a 0.6% phosphorus diet. At least 8 days were allowed to elapse over which weight gain was documented before experiments were initiated. These PTX rats were divided into six groups as follows:

Group 1: effects of somatostatin on PO_4 excretion (N = 12). Clearance experiments were performed using standard techniques as previously described [5, 6] during sustained infusion with sodium chloride (150 mM containing 6 mg% Ca) at a rate of 10 ml/100 g/hr. This degree of extracellular fluid volume expansion was felt necessary to rapidly achieve a relatively steady state to minimize time-associated changes in tubular fluid and sodium handling in the course of recollection micropuncture experiments that lasted 5 to 6 hr (see groups 5 and 6 below).

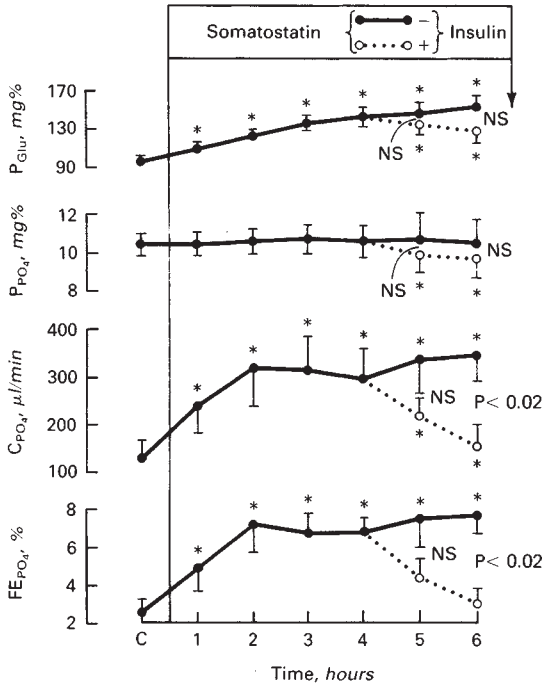

Fig. 1. *Effects of somatostatin on plasma and clearance data.* Abbreviation and symbol are: C, basal phase before somatostatin; *, indicates significant difference of $P < 0.01$ compared to the basal phase. P values given refer to differences due to the presence of concomitant insulin; NS, not significant.

After equilibration for 90 min, four 20-min urine collections were made for the basal phase. In the succeeding experimental phase, an analogue of somatostatin, des-Asn[5]-D-Trp[8]-D-Ser[13] (kindly supplied by Drs. J. Rivier and M. Brown of Salk Institute, LaJolla, California), known to be highly selective in suppressing insulin [7, 8], was infused at 10 µg/kg/hr in the same infusate for 4 hr. After that, in half of the group, the somatostatin was continued for 2 more hr, while in the remaining half, regular insulin (Iletin, Eli Lilly, Indianapolis, Indiana) was added in the infusate to deliver 0.1 U/kg/hr for 2 hr. Arterial blood and urine specimens were obtained for the determination of clearance of inulin, PO_4, and sodium. Blood glucose was also monitored hourly throughout the experiment.

Group 2: role of hyperglycemia (N = 6). Plasma glucose increased progressively during the suppression of insulin by somatostatin infusion (Fig. 1) from 93 to a steady level of 137 mg% (third hour) and 143 mg% (fourth hour). Because glucose in blood [3] and the tubules [9, 10] has been shown to alter PO_4 reabsorption, independent of plasma insulin, this variable was examined by infusing dextrose at 400 mg/kg/hr to achieve a similar degree of hyperglycemia in the 4-hr experimental phase. Similar clearance studies were performed as in group 1.

Group 3: role of increased natriuresis (N = 6). Fractional excretion of sodium rose slightly during somatostatin infusion from a basal value of 11.1% to reach a steady level of 13.5% (see below). To evaluate the role of this factor, after a basal phase similar to group 1, the infusion of saline was increased to 15 ml/100 g/hr over the 4-hr experimental phase.

Group 4: role of plasma insulin. Basal insulin levels (N = 12). Rats were treated identically to those in group 1. Carotid blood was drawn directly in ice-chilled containers (six after 40 min and six after 80 min into the basal phase) for determination of plasma insulin. Glucagon was also measured in four of them.

Insulin during somatostatin suppression (N = 15). Rats were treated identically as those in group 1. Carotid blood was drawn (five after 2 hr, five after 4 hr, and five after 6 hr of somatostatin infusion) for insulin determination. Glucagon was also measured in four of them.

Insulin levels during combined infusion of somatostatin and insulin (N = 12). Rats were treated identically to those in group 1 through the sixth hour of somatostatin infusion, over the last 2 hr of which insulin was added. Carotid blood was then obtained for insulin determination.

Group 5: saline vehicle—time control (N = 6). Two-phase (90 to 120 min each) recollection micropuncture studies were performed by techniques previously described [5] during sustained infusion of the saline vehicle at rates identical to those in group 1.

Group 6: effects of somatostatin on tubular PO_4 transport (N = 6). This group was studied similarly to group 5 except for the addition of somatostatin (10 µg/kg/hr) 30 min before and throughout the 2-hr recollection (experimental) phase.

Specimen analysis and calculations

^{3}H-inulin in plasma, urine, and tubular fluid was analyzed by liquid scintillation counter (Hewlett-Packard, Palo Alto, California). Calcium, PO_4, and sodium in plasma and urine were determined by methods previously described [5, 6]. Tubular fluid PO_4 was analyzed by an electron microprobe (ARL). Except for the substitution for the Cameca model, details of sample processing and analyses were identical to those in published reports [5, 11]. Unknowns were accepted only if correlation coefficients between micro (by the probe) and macro analysis (by the conventional phosphomolybdate method described above) exceeded 0.99. Insulin and glucagon were determined by the laboratory of the University of Michigan Diabetes Research and Training Center using the double antibody RIA techniques previously described [12]. Glucose was measured by the glucose oxidase method.

Standard formulae were used for the calculations of clearance of insulin (C_{In}), sodium, and PO_4 and for the fractional excretion (FE) of sodium and PO_4. Fractional delivery (FD) of PO_4 was calculated by the ratio of tubular fluid (TF) to plasma ultrafiltrable (UF) PO_4 over that for inulin. All results are expressed as mean ± SEM. Data were subject to statistical analysis using the Student's t test, paired or nonpaired as appropriate. Differences were considered significant if P values were less than or equal to 0.05.

Results

Group 1: effects of somatostatin (Fig. 1). There was no change in GFR during the 4 hr of somatostatin infusion 4.6 ± 0.2 (basal), 4.7 ± 0.3 (first hour), 4.6 ± 0.3 (second hour), and 4.5 ± 0.3 (third hour), 4.4 ± 0.3 ml/min (fourth hour). This parameter remained stable in the succeeding 2 hr whether insulin was added (4.8 ± 0.4 and 4.7 ± 0.2 ml/min) or not (4.5 ± 0.3 and 4.6 ± 0.4 ml/min). FE_{Na} rose from 11.1 ± 0.9 to 13.5 ± 0.8 (second hour), 13.4 ± 0.8 (third hour), and 13.8 ± 0.7% (fourth hour) ($P < 0.05$ for all). From a basal level of 93 mg%, plasma glucose rose to 109 (first hour), 123 (second hour), 137

Table 1. Effects of loading with saline and glucose versus somatostatin infusion (mean ± SE)

	Plasma PO_4 *mg%*			Plasma glucose *mg%*			GFR *ml/min*		C_{PO_4} *μ liters/min*			FE_{PO_4} %			FE_{Na} %		
	B	E	Δ	B	E	Δ	B	E	B	E	Δ	B	E	Δ	B	E	Δ
Saline[a] Loading (N = 6)	10.9 ±0.4	10.7 ±0.5	−0.25 ±0.13	—	—	—	3.8 ±0.3	4.0 ±0.4	140 ±37	74[d] ±20	−68 ±26	3.8 ±1.1	1.9 ±0.7	−2.0 ±0.9	10.4	13.3[c] ±1.1	2.9 ±0.3
P value[b]	NS	NS	NS				< 0.05	NS	NS	< 0.03	< 0.001	NS	< 0.03	< 0.001	NS	NS	NS
Somatostatin[a] (N = 12)	10.4 ±0.6	10.5 ±0.7	0.14 ±0.32	93 ±5	140 ±7	47 ±6	4.6 ±0.2	4.5 ±0.2	118 ±41	297[c] ±63	180 ±35	2.4 ±0.8	6.8[c] ±1.3	4.4 ±0.8	11.1 ±0.9	13.6[d] ±0.7	2.5 ±1.0
P value[b]	NS	NS	NS	NS	NS	NS	NS	< 0.03	NS	< 0.05	< 0.001	NS	< 0.05	< 0.001			
Glucose[a] Loading (N = 6)	11.4 ±0.3	10.7 ±0.4	−0.72 ±0.62	91 ±17	143 ±4	52 ±10	3.9 ±0.4	3.7 ±0.2	150 ±28	88[d] ±18	−58 ±14	3.9 ±0.8	2.2[d] ±0.4	−1.9 ±0.6	—	—	—

Abbreviations: GFR, glomerular filtration rate; C_{PO_4}, clearance of PO_4; FE_{PO_4} and FE_{Na}, fractional excretion of PO_4 and Na; B, basal phase; E, experimental phase.

[a] Mean values from the third and fourth hours of infusion with saline, SRIF, or glucose.

[b] P values refer to comparison between groups above and below.

[c] $P < 0.001$ vs. basal within the same group.

[d] $P < 0.05$ vs. basal within the same group.

Table 2. Effects of somatostatin on plasma and clearance data (mean ± SE)

		GFR *ml/min*	Plasma UF PO_4 *mg%*	C_{PO_4} *μliters/min*	FE_{PO_4} %
Time Control (N = 6)	B	3.2 ±0.4	8.9 ±0.8	140 ±29	4.4 ±0.7
	E	3.1 ±0.3	9.2 ±0.6	112 ±31	3.5 ±0.8
P value[a]		NS	NS	NS	NS
Somatostatin (N = 6)	B	2.9 ±0.3	9.1 ±1.0	128 ±47	4.9 ±2.1
	E	3.0 ±0.4	9.0 ±0.8	257 ±53	8.5 ±1.8
P value[a]		NS	NS	<0.05	<0.05

Abbreviations: UF, ultrafiltrable; other abbreviations are found in Table 1.

[a] P values refer to differences between basal and experimental phases within a given group.

(third hour), and 143 mg% (fourth hour) in response to somatostatin. There was no further change over the next 2 hr or any significant difference between the two subgroups with or without additional insulin. Plasma PO_4 was stable throughout the 6 hr of somatostatin infusion. The addition of insulin was associated with a slight fall to 9.9 ± 0.9 and 9.5 ± 0.9 mg%, but these values were not different from those rats that did not receive insulin (10.5 ± 1.5 and 10.3 ± 1.4 mg%). C_{PO_4} rose from 118 to between 231 and 311 μl/min. Continued somatostatin infusion was associated with a sustained increase in C_{PO_4} 330 (fifth hour) and 342 μl/min (sixth hour) whereas the added insulin restored C_{PO_4} to baseline level 212 (fifth hour) and 146 μl/min (sixth hour). During the last hour of insulin infusion, C_{PO_4} was significantly lower than those rats receiving only somatostatin (Fig. 1). FE_{PO_4} similarly rose during somatostatin infusion (increasing from 2.4 to between 4.8 and 7.1%), which was reduced by insulin to 2.9% (vs. 7.6 in the subgroup not receiving insulin) (Fig. 1). Urine glucose by dextrostix (Miles Laboratories, Elkhart, Indiana) was all negative.

Groups 2 and 3: role of hyperglycemia and increased natriuresis (Table 1). To exclude the role of hyperglycemia and natriuresis, the effects of loading separately with saline and glucose were compared to somatostatin. There was no change in plasma PO_4 or GFR in response to saline loading (Table 1). At comparable increments of FE_{Na} (2.9 vs. 2.5 for somatostatin), there was a slight fall in C_{PO_4} (−68 μl/min) or no change in FE_{PO_4} (−2.0 ± 0.9%) versus increases associated with somatostatin. Glucose loading was associated without significant alterations in plasma PO_4 or GFR. At comparable increments of plasma glucose (52 vs. 47 mg% for somatostatin), there was a slight fall in both C_{PO_4} (−58 ± 14 μl/min) and FE_{PO_4} (−1.9 ± 0.6%) versus the increments in somatostatin-infused rats. These data indicate that the phosphaturia during somatostatin administration was not mediated by the concomitant hyperglycemia or natriuresis.

Group 4: role of plasma insulin levels. The basal level of insulin, comparable whether obtained 40 or 80 min into the basal phase, was 12.6 ± 3.5 U/ml. Somatostatin infusion reduced the concentration markedly and by similar degrees after 2, 4, or 6 hr, to 2.8 ± 1.0 U/ml, $P < 0.01$. The addition of exogenous insulin ($P < 0.01$) increased the plasma level to 30.6 ± 12 U/ml, which was not significantly different from the basal value. The glucagon level was 28 ± 9 pg/ml in the basal period and was not significantly altered by somatostatin (41 ± 10 pg/ml).

Groups 5 and 6: tubular effects of somatostatin on PO_4 transport (Tables 2 and 3). Between the basal and experimental phases there was no change in both the time control and somatostatin infused groups for GFR and plasma ultrafiltrable PO_4 (Table 2). While C_{PO_4} and FE_{PO_4} remained stable in the time control, these parameters rose in response to somatostatin infusion (128 to 257 μl/min and 4.9 to 8.5%, respectively; Table 2).

Table 3. Effects of somatostatin on segmental fluid and PO_4 transport (mean ± SE)

		Late proximal			Early distal			Late distal		
		$\frac{TF}{P}In$	$\frac{TF}{UF}PO_4$	FD_{PO_4} %	$\frac{TF}{P}In$	$\frac{TF}{UF}PO_4$	FD_{PO_4} %	$\frac{TF}{P}In$	$\frac{TF}{UF}PO_4$	FD_{PO_4} %
Time	B	1.87	0.63	34	3.3	0.51	15.0	6.4	0.46	7.5
Control		±0.13	±0.12	±4	±0.2	±0.09	±3.8	±0.6	±0.08	±0.9
(N = 6)	E	1.90	0.69	37	3.1	0.52	16.5	6.7	0.47	7.1
		±0.24	±0.15	±4	±0.3	±0.10	±3.6	±0.7	±0.13	±2.0
P values[a]		NS	NS	NS	NS	NS	NS	NS	NS	NS
Somatostatin	B	1.91	0.60	32	3.4	0.48	13.4	6.8	0.43	6.4
(N = 6)		±0.12	±0.14	±3	±0.4	±0.12	±4.3	±1.2	±0.17	±2.8
	E	1.86	0.93	50	3.3	0.91	28.2	7.4	0.86	11.8
		±0.10	±0.09	±4	±0.4	±0.15	±5.7	±1.5	±0.23	±2.5
P values[a]		NS	<0.02	<0.02	NS	<0.01	<0.005	NS	<0.05	<0.05

Abbreviations: In, inulin; FD_{PO_4}, fractional delivery of PO_4; other abbreviations are found in Table 1.
[a] P values refer to differences between basal and experimental phases within a given group.

Similarly, between the basal and experimental phases, there was no significant difference in TF/P inulin at all three points of micropuncture in both the time control and the somatostatin-treated rats (Table 3). TF/UF PO_4 and FD_{PO_4} were unchanged in the time control group. However, somatostatin increased TF/UF PO_4 (0.60 to 0.93) and FD_{PO_4} (32 to 50%) in the late proximal tubular fluid, indicating an inhibition of PO_4 transport in the proximal convoluted tubule. Similar increases in TF/UF PO_4 and FD_{PO_4} were noted for the early distal puncture (0.48 to 0.91 and 13.4 to 28.2%) and late distal puncture (0.43 to 0.86 and 6.4 to 11.8%, respectively), indicating that the proximal inhibition was still detectable in the distal nephron.

Plasma ultrafiltrable calcium (mg %) fell slightly but significantly in all groups (group 1, 3.76 to 3.42; group 2, 3.78 to 3.45; group 3, 3.85 to 3.5; group 5, 3.73 to 3.44; and group 6, 3.77 to 3.49; group 4, not measured) consistent with the chronic PTX and the volume-expanded conditions of our preparation. But the magnitudes of fall were comparable among all groups measured.

Discussion

The results of the present investigation show that the infusion of des-Asn^5-D-Trp^8-D-Ser^{13} (d-ATS-SS), an analogue of somatostatin highly selective for insulin, produces a phosphaturia in the absence of PTH or changes in plasma PO_4 (Fig. 1 and Table 2). Furthermore, the tubular fluid data suggest that PO_4 transport is inhibited in the proximal convoluted tubule (Table 3), exhibiting effects that are independent of fluid reabsorption and sustained in the distal nephron. It was not possible to attribute the reduced PO_4 reabsorption to increased natriuresis (ΔFE_{Nd} of 2.5%) since similar increments in sodium excretion (2.9%) could not reproduce the phosphaturic effects (Table 1).

Previous work [13] revealed no significant effects of somatostatin on the phosphaturic or hypocalciuric action of PTH in PTX dogs. Their negative results could not be attributed to differences in species [14] or dosages. There are two possible explanations. First, their studies [13] were not primarily designed to examine the effects of somatostatin, since the infusion lasted only 20 min and since no direct comparison between control and somatostatin was made. Second, their somatostatin preparations [13] also suppressed other peptides, such as glucagon, recently shown to affect renal PO_4 reabsorption [15]. Thus, changes in glucagon level (not determined) might obscure a net effect on renal PO_4 handling, especially in the presence of PTE infusion.

In considering the mechanism for the effects of d-ATS-SS on tubular PO_4 transport, several possibilities must be included: *(1)* somatostatin per se versus the secondary effects of somatostatin, viz., *(2)* changes in adenylate cyclase-cyclic adenosine monophosphate (cAMP), *(3)* hyperglycemia, *(4)* hyperglucagonemia, and *(5)* insulinopenia. Some of these potential mediators can be readily dismissed for the lack of evidence.

Thus, there is no demonstrable effect of somatostatin on cAMP concentration or release in rat hepatocytes [16], colon [17], rabbit ileum [18], or human urine [14], despite earlier studies in chick renal cells [19]. Although these conflicting data may simply reflect differences in species, the fact that our findings were made in PTX rats makes it highly unlikely that changes in PTH sensitive adenylate cyclase and/or cAMP are responsible for the phosphaturic effects of somatostatin infusion. This impression is reinforced by the observations that somatostatin exerted no significant influence on the phosphaturic or hypocalciuric effects of PTE [13].

Previous clearance studies in intact dog [20–22] and man [23–25] suggested an inhibitory effect of hyperglycemia on PO_4 reabsorption, but the role of glycosuria could not be dissociated. In the dog studies [20–22], data interpretation was further complicated by the hyperphosphatemia. Direct demonstration of intratubular glucose on proximal tubule PO_4 transport was recently made by micropuncture studies [3], microinjection studies [9], and microperfusion experiments [10]. Again, when glycosuria was carefully prevented by employing only subthreshold hyperglycemia, urinary PO_4 excretion fell [3], indicating an enhancement of transport by this maneuver distal to the proximal convolution.

Glucose loading to reproduce the mild increment in plasma glucose (Δ = 52 vs. 47 in the somatostatin experiments) reduced PO_4 excretion when glycosuria was absent, confirming the observations of DeFronzo, Goldberg, and Agus [3] and supporting the thesis that glucose increases overall renal PO_4 reabsorption despite the proximal inhibition. Therefore, although the proximal tubule effects of somatostatin could be due to mild

elevation in plasma glucose, the sustained inhibition in the distal nephron by somatostatin could only be mediated by factors other than glucose.

Two, the somatostatin-induced inhibition of proximal PO_4 transport was not associated with diminished fluid reabsorption, in contrast to the inhibitory effects of subthreshold hyperglycemia [3]. Three, plasma glucose was equally elevated between the 4-hr "somatostatin" phase and the 2-hr "somatostatin + insulin" phase in those six animals infused with insulin (129 ± 11 vs. 134 ± 10 mg%). Yet mean FE_{PO_4} was reduced significantly in the presence of insulin (6.4 ± 1.3 to 3.5 ± 0.8%, $P < 0.05$). Finally, the inhibition by glucose was only observed when differences in glucose concentration in the plasma [3, 9] and/or tubular lumen [9] reached or exceeded 70 [3] to 110 mg% [9], magnitudes two- to threefolds greater than that produced by SRIF. Similar differences were found necessary to retard PO_4 uptake by renal brushborder membrane vesicles [26]. In the isolated rabbit tubule studies by Dennis and Brazy [10], although the presence of glucose retards lumen to bath PO_4 uptake, no effect could be demonstrated over the range of glucose concentrations from 108 to 216 mg%. Thus, the weight of the evidence suggests that changes in plasma insulin best explains the inhibition of tubular PO_4 transport by somatostatin (Table 3), and the enhancement of distal nephron PO_4 reabsorption by the hyperinsulinemia [3]. Nevertheless, it should be acknowledged that we could not definitely exclude the possibility of a contributory role of hyperglycemia in the phosphaturia of somatostatin.

Since glucagon in pharmacological doses is phosphaturic [1, 15], associated with inhibition of PO_4 transport in the proximal tubule [27], it is possible that the effects of d-ATS-SS were mediated by glucagon which is not suppressed by our selective analogue glucagon. However, plasma glucagon did not change significantly (28 to 41 pg/ml) in our experiments, as was shown recently in dogs similarly treated [8].

Recent in vitro studies suggest the stimulation of renal gluconeogenesis (GNG) by somatostatin, that can be abolished by insulin [28]. Since the rate of GNG has been postulated to affect proximal tubular PO_4 transport [29] via changes in the ratio of oxidized nicotinamide adenine dinucleotide (NAD^+) to the reduced form NADH [30, 31], the tubular action of somatostatin could be explicable by the metabolic effects on GNG. Indeed, the sites of inhibition by somatostatin correspond to the major loci of gluconeogeneic activity [32]. However, this thesis is presently rather speculative without additional studies simultaneously measuring transport and metabolism, in view of recently reported conflicting experimental data [33, 34].

While the intracellular or biochemical mediator for the tubular effects of somatostatin could not be identified by the present studies, the data nevertheless are strongly suggestive of a role for insulin. First, hyperinsulinemia within the physiologic range (35 to 80 μU/ml) due to endogenous release [3] or exogenous infusion [1–4] has been shown to increase whole kidney PO_4 reabsorption, the mirror image of the phosphaturic effects of insulinopenia (2.8 vs. 12.6 μU/ml) produced by somatostatin. Contrary to previous studies [1–4], where PO_4 retention could be attributed to an absolute or relative fall [3] in plasma or filtered PO_4, our experiments excluded the role of this variable (Fig. 1 and Table 2). Our tubular fluid data indicate that deficiency of insulin, independent of PTH, impairs proximal tubule PO_4 transport (Table 3), the converse of the augmented proximal PO_4 reabsorption by insulin [3]. Second, when insulin was replaced in the clearance studies, the phosphaturia was abolished despite sustained infusion of somatostatin (Fig. 1). Since plasma glucose remained elevated (136 and 132 vs. 93 mg% in the basal phase), changes in plasma insulin were the only variable altered that could be implicated to reverse the effects of somatostatin, in contrast to the comparable degree of hyperglycemia and constant somatostatin infusion.

Third, preliminary reports [35] also suggest enhanced PO_4 uptake by luminal brushborder membrane vesicles from dogs infused with insulin. It should be stated that conceivably both somatostatin and insulin could separately modify PO_4 reabsorption, perhaps via some common denominator(s), such as the rates of GNG and/or the ratio of NAD^+/NADH, since depressant effects of insulin on renal GNG [28, 36] and intracellular NAD^+/NADH ratio [37] have been reported.

It is not possible to establish a direct effect of insulinopenia on PO_4 transport in the pars recta because of the increased proximal rejection (FD_{PO_4} = 50 vs. 32%) (Table 3). Recent micropuncture data evaluating the response of this tubular segment to volume expansion indicate that PO_4 transport is load-dependent [38]. Sustenance of the proximal inhibition in the early distal tubular fluid (FD_{PO_4} = 28.2 vs. 13.4%) is consistent with impaired transport by the somatostatin in the pars recta (Table 3). In addition, as a percentage of the delivered load, reabsorption by this tubular segment was depressed by somatostatin (43 vs. 58%). Though circumstantial, these results suggest a tubular mechanism for the antiphosphaturic action of hyperinsulinemia (due to subthreshold hyperglycemia) despite the proximal inhibition [3].

Although the role of glycosuria (and osmotic diuresis), calciuria, and changes in PTH and glucagon could not be dissociated, chronic insulin therapy in the diabetic human has recently been shown to be associated with a positive PO_4 balance via diminished renal PO_4 clearance [39, 40].

In summary, selective insulinopenia produced by the infusion of a somatostatin analogue is associated with impaired PO_4 transport in the proximal convoluted (and probably the straight) tubules and increased phosphaturia. These findings are consistent with the concept that basal levels of endogenous insulin are important in renal PO_4 homeostasis independent of PTH, plasma glucose, and PO_4 concentrations.

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Reprint requests to Dr. K. Lau, Division of Nephrology, Department of Internal Medicine, The University of Michigan, D3238 South Ambulatory Care Building, Ann Arbor, Michigan 48109, USA

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